Contralateral fatigue during severe-intensity single-leg exercise: influence of acute acetaminophen ingestion

Original investigation

Paul T. Morgan[1], Stephen J. Bailey[1,3], Rhys A. Banks[1], Jonathan Fulford[2], Anni Vanhatalo[1], and Andrew M. Jones[1]

[1] Department of Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, EX1 2LU, UK.

[2]Peninsula NIHR Clinical Research Facility, College of Medicine and Health, Exeter, UK.

Address for Correspondence:
Professor Andrew Jones
Department of Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, EX1 2LU, UK.
Tel: 01392 722 886
E-mail: A.M.Jones@exeter.ac.uk

[3]Present address for Stephen J. Bailey: School of Sport, Exercise and Health Sciences, Loughborough University, Epinal Way, Loughborough, Leicestershire LE11 3TU

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ABSTRACT

Exhaustive single-leg exercise has been suggested to reduce time to task failure (T_{lim}) during subsequent exercise in the contralateral leg by exacerbating central fatigue development. We investigated the influence of acetaminophen (ACT), an analgesic which may blunt central fatigue development, on T_{lim} during single-leg exercise completed both with, and without, prior fatiguing exercise of the contralateral leg. Fourteen recreationally-active men performed single-leg, severe-intensity knee extensor exercise to T_{lim} on the left (Leg_1) and right (Leg_2) legs without prior contralateral fatigue, and on Leg_2 immediately following Leg_1 ($Leg_{2\text{-CONTRA}}$). The tests were completed following ingestion of 1 g ACT or maltodextrin (placebo) capsules. Intramuscular phosphorous-containing metabolites and substrates, and muscle activation, were assessed using ^{31}P-MRS and electromyography, respectively. T_{lim} was not different between the Leg_{1ACT} and Leg_{1PL} conditions (402 ± 101 vs. 390 ± 106 s; P>0.05). There was also no difference in T_{lim} between $Leg_{2ACT\text{-}CONTRA}$ and $Leg_{2PL\text{-}CONTRA}$ (324 ± 85 vs. 311 ± 92 s; P>0.05), but T_{lim} was shorter in these tests compared to Leg_{2CON} (385 ± 104 s; P<0.05). There were no differences in intramuscular phosphorous-containing metabolites and substrates, or muscle activation, between the Leg_{1ACT} and Leg_{1PL} or the $Leg_{2ACT\text{-}CONTRA}$ and $Leg_{2PL\text{-}CONTRA}$ conditions (P>0.05). These findings suggest that task failure during single-leg severe-intensity knee extensor exercise is associated with the attainment of a similar level of metabolic perturbation and muscle activation, both with and without prior fatiguing exercise of the contralateral leg. Despite the existence of contralateral fatigue, ACT ingestion did not alter neuromuscular responses or exercise performance.

Key words: ^{31}P-magnetic resonance spectroscopy; intramuscular metabolites; intramuscular substrates; non-local muscle fatigue, paracetamol

INTRODUCTION

The mechanisms of exercise-induced fatigue can be attributed to processes within the central nervous system, termed central fatigue, and within the contractile elements of the working muscle, termed peripheral fatigue. It is now recognised that peripheral and central fatigue development are interlinked, in part, via group III/IV muscle afferent feedback (25). Empirical support for a role of group III/IV muscle afferent feedback in modulating the mechanisms of neuromuscular fatigue is provided by reports that inhibiting group III/IV muscle afferent feedback, via lumbar intrathecal administration of fentanyl, lowers central fatigue development and results in increased skeletal muscle metabolic perturbation [greater and/or more rapid increases in adenosine diphosphate (ADP) and inorganic phosphate (Pi) accumulation and declines in phosphocreatine (PCr) and pH] and thus peripheral fatigue development (i.e., 1, 2, 8, 10, 11, 12, 38, 39, 40). Conversely, prior fatiguing single-limb exercise has been reported to accentuate central fatigue development and lead to lower peripheral fatigue development during subsequent fatiguing exercise in a contralateral or non-local (previously rested) muscle group, when group III/IV muscle afferent feedback would be expected to be elevated (3, 22, 23, 26, 33, 40). However, the underlying mechanisms of non-local muscle fatigue, including the effect of prior fatiguing single-limb exercise on skeletal muscle metabolic perturbation during subsequent fatiguing exercise in a contralateral or non-local muscle group, have yet to be resolved (ref. 23 *for review)*. Moreover, while lumbar intrathecal administration of fentanyl and prior fatigue of a contralateral or non-local muscle group can alter group III/IV muscle afferent feedback and the physiological bases of exercise-induced neuromuscular fatigue, the effect of such interventions on exercise performance is equivocal (i.e., 1, 2, 3, 8, 10, 11, 12, 22, 23, 26, 28, 33).

There is an emerging body of evidence to suggest that oral ingestion of acetaminophen (ACT) can blunt the development of exercise-induced neuromuscular fatigue and improve exercise capacity and/or performance (19, 30, 31, 32). It is generally accepted that the principal mechanism of action of ACT is the inhibition of cyclooxygenase, the enzyme that catalyses the synthesis of prostaglandins from arachidonic acid (4). Since prostaglandins sensitize nociceptors (36, 37), and since blocking cyclooxygenase attenuates group III/IV muscle afferent discharge during dynamic exercise (24), this might account for reports of increased work output for the same level of perceived pain and exertion (19, 30), and elevated muscle activation (31, 32), during exercise after ACT ingestion. Therefore, ACT administration might be ergogenic by reducing, but not abolishing, the net magnitude of group III/IV muscle afferent feedback, leading to a blunting of exercise-induced central fatigue. Since ACT appears to attenuate exercise-induced neuromuscular fatigue by abating aspects of central fatigue development (19, 30, 31, 32), ACT might be more effective at lowering exercise-induced neuromuscular fatigue following prior exhaustive exercise in a contralateral limb. However, the effects of ACT ingestion on exercise-induced fatigue development and its underlying mechanisms following prior exercise in a contralateral limb have yet to be investigated.

The purpose of this study was to investigate the effects of ACT ingestion on exercise-induced neuromuscular fatigue and some of its underlying mechanisms during single-leg severe-intensity knee extensor exercise completed with and without prior exhaustive severe-intensity knee extensor exercise in the contralateral leg. It was hypothesised that: 1) prior exhaustive exercise would impair subsequent exercise tolerance in the contralateral leg by lowering muscle activation and the degree of muscle metabolic perturbation [changes in muscle pH and PCr ([PCr]), ADP ([ADP]) and Pi ([Pi]) concentrations] that could be attained; 2) ACT ingestion would enhance single-leg knee extensor exercise tolerance by increasing muscle

activation (higher surface EMG) and permitting the attainment of a greater degree of muscle metabolic perturbation; and 3) ACT ingestion would improve exercise tolerance to a greater extent with, compared to without, the completion of prior exercise by the contralateral leg.

MATERIALS AND METHODS

Subjects

Fourteen active males volunteered to participate in this study (mean ± SD: age 23.8 ± 4.7 y, height 1.80 ± 0.10 m, body mass 81.6 ± 14.9 kg). All procedures were approved by the Ethics Committee of the Department of Sport and Health Sciences, University of Exeter. This study conformed to the principles of the World Medical Association Declaration of Helsinki. Subjects completed a health questionnaire, which was checked by a medical doctor, to ensure it was safe to consume ACT prior to performing exhaustive exercise. The questionnaire incorporated questions pertaining to: known allergies to medications, current intake of medication and prior use of ACT as well as any history of illnesses, cigarette and illegal drug use, alcohol consumption, and chronic illnesses (personal and family history). Prior to each visit, subjects were required to refrain from caffeine (for at least 12 h), strenuous exercise and alcohol (for at least 24 h), analgesics and any form of anti-inflammatory drug (for the duration of the experiment) and to arrive in a fully rested, hydrated state. With the exception of these restrictions, subjects were instructed to maintain their usual diet and exercise regime during the study. All tests were performed at a similar time of day (± 2 h).

Pre-experimental procedures

Subjects visited the laboratory on twelve occasions over an 8-12 week period to complete the experimental testing, with a minimum of 72 h separating all tests (figure 1). The experimental testing incorporated 4 pre-experimental trials (visits 1-4) and 8 experimental trials (visits 5-

12). *Visits 1-4* were completed within a replica of an MRI scanner (with no magnetic field present). Initially, subjects completed a single-limb incremental test on the left leg (*visit 1,* Leg_1) and right leg (*visit 2,* Leg_2) to task failure to establish the limb-specific work rates that would be applied in subsequent experimental visits (as described below). Following these preliminary tests, subjects completed a familiarisation session on *visits 3 and 4* which comprised a single-leg severe-intensity constant work rate (CWR) test to task failure with the left leg (Leg_1), a single-leg severe-intensity CWR test to task failure with the right leg (Leg_2), and a crossover test where the Leg_1 protocol was repeated and immediately followed by the Leg_2 protocol to assess contralateral fatigue in Leg_2. In these preliminary tests, the Leg_1, Leg_2 and Leg_2 contralateral protocols were interspersed by 10 min of passive recovery.

Experimental procedures

During *visits 5 and 6*, subjects completed the Leg_1 and Leg_2 protocols without oral consumption of any capsules (Leg_{1CON} and Leg_{2CON}, respectively). On *visits 7 and 8*, subjects completed the crossover limb tests described above, 45 mins following the consumption of 1 g maltodextrin (placebo, PL) to determine time to task failure (T_{lim}) values for Leg_1 (Leg_{1PL}) and Leg_2 contralateral ($Leg_{2PL\text{-}CONTRA}$), and 45 mins following the consumption of 1 g ACT, to determine T_{lim} values for Leg_1 (Leg_{1ACT}) and Leg_2 contralateral ($Leg_{2ACT\text{-}CONTRA}$). PL and ACT were administered in the form of 2 identically coloured pills. The placebo was made from maltodextrin powder inserted into gelatine capsules designed to have a similar appearance to ACT without inducing any analgesic or antipyretic effects. The oral consumption of PL and ACT ~45 min prior to commencing exercise was selected to broadly coincide with attainment of the peak plasma [ACT], which occurs ~60 min post ACT ingestion (4, 17), at the onset of the $Leg_{2\text{-}CONTRA}$ tests. The PL and ACT conditions were administered double-blind in a counterbalanced cross-over experimental design. *Visits 5-8* were completed within the bore of

an MRI scanner for assessment of exercise-induced changes in intramuscular phosphorous-containing substrates and metabolites. *Visits 5-8* were replicated in *visits 9-12* within a replica of the MRI scanner (with no magnetic field present) to assess muscle electromyography (EMG) and ratings of perceived exertion (RPE).

Experimental set-up

Exercise tests were performed in a prone position within the bore of a 1.5 T superconducting magnet (Gyroscan Clinical Intera, Philips, The Netherlands) using a custom-built ergometer for the assessment of intra-muscular [PCr], [Pi], [ADP] and pH (*visits 5-8*) or within a replica of the MRI scanner for preliminary testing (*visits 1-4*) and the assessment of EMG and RPE responses (*visits 9-12*). Subjects' feet were fastened securely to padded foot braces using Velcro straps and connected to the ergometer load baskets via a rope and pulley system. The sprocket arrangement was such that when a bucket containing non-magnetic weights was attached, it provided a concentric-only resistive load, allowing for the performance of rhythmic knee-extension exercise. Single-leg knee-extensions over a distance of ~0.22 m were performed continuously at a constant frequency which was set in unison with the magnetic pulse sequence (40 pulses min^{-1}) to ensure the quadriceps muscle was in the same phase of contraction during each magnetic resonance pulse acquisition. To prevent displacement of the quadriceps relative to the magnetic resonance spectroscopy (MRS) coil, Velcro straps were also fastened over the subject's thighs, hips and lower back.

Experimental protocol

To determine peak work rate (WR_{peak}) for each leg, subjects initially completed single-leg incremental knee-extensor exercise on *visits 1 and 2* until they were unable to continue the prescribed work rate, as described previously (43). The load for the initial increment was 4 kg

149 and this was increased by 0.5 kg·min^{-1} thereafter until T_{lim}. T_{lim} was recorded when subjects
150 were unable to sustain the required contraction frequency for 3 consecutive repetitions.
151 Following these initial tests, subjects were familiarized with the different exercise tests that
152 comprised the experimental testing protocol. During these visits, a limb-specific, high-intensity
153 work rate, which was expected to elicit T_{lim} in approximately 5–8 min, was prescribed for each
154 subject.
155
156 The experimental exercise protocol consisted of CWR, single-leg knee-extension to T_{lim}.
157 Initially, subjects completed single-leg knee-extension exercise for each limb individually over
158 two separate laboratory visits. Subsequently, to investigate the influence of ACT on
159 contralateral leg fatigue, subjects completed single-leg knee-extension exercise until task
160 failure with Leg_1, followed consecutively (<3 s) by the identical task with the contralateral leg
161 (i.e., Leg_2). These crossover tests to assess contralateral fatigue in Leg_2 were completed 60 min
162 following the consumption of PL and ACT over two separate laboratory visits. For all trials,
163 subjects received strong verbal encouragement to continue for as long as possible but no
164 feedback was given on the elapsed time.
165
166 *MRS measurements*
167 ^{31}P-MRS data were acquired every 1.5 s with a spectral width of 1,500 Hz and 1,000 data
168 points. Phase cycling with four phase cycles was used, leading to a spectrum being acquired
169 every 6 s. The subsequent spectra were quantified by peak fitting, using the AMARES fitting
170 algorithm in the jMRUI (v3) software package. Absolute values of [PCr] and [Pi]
171 concentrations were subsequently calculated via the ratio of PCr:adenosine triphosphate (ATP)
172 and Pi:ATP assuming an ATP concentration of 8.2 mM. Intracellular pH was calculated using
173 the chemical shift of the Pi spectra relative to the PCr peak. The ADP concentration was

calculated as described by Kemp *et al*. (27). In all cases, relative amplitudes were corrected for partial saturation resulting from the short repetition time relative to T1 relaxation time, via a spectrum consisting of 24 averages that was acquired with a TR of 20 s prior to the comencement of exercise testing.

Electromyography

Throughout *visits 9-12*, muscle activity of the right and left *m.*vastus lateralis was recorded using active bipolar bar electrodes with a single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA). Initially, the leg was shaved and cleaned with alcohol to minimize skin impedance. The electrodes were placed over the respective muscle bellies parallel to the longitudinal axis of each muscle (SENIAM guidelines). Double-sided adhesive tape and a hypoallergenic medical tape were used to ensure the EMG sensor stability. The position of the EMG electrodes was measured with respect to the location of the patella and the anterior superior iliac spine and marked with indelible ink to ensure placement in the same location on subsequent visits. The ground electrode was placed over the patella of the respective leg. The EMG signals were pre-amplified (1,000x), band-pass filtered (20–450 Hz, Bagnoli-8, DelSys Inc, Boston, MA, USA), and then transferred to a computer with a sampling frequency of 2 kHz. EMG data were recorded continuously and digitised synchronously with 16 bit resolution via an A/D converter (± 5 V range, CED 1401 power, Cambridge, UK) using Spike2 software (CED, Cambridge, UK). During these trials, ratings of perceived exertion (RPE) was measured at 2-min intervals from the onset of exercise using Borg's 6-20 scale (9).

Data Analysis

Baseline values for [PCr], [P_i], [ADP], and pH were defined as the mean values measured over the final 60 s of rest (i.e., prior to initiation of the severe-intensity exercise bout). Baseline

values for Leg_2 during the crossover protocol (for both PL and ACT) were calculated during the final 60 s of exhaustive Leg_1 exercise. End-exercise values for these variables were defined as the mean values measured over the final 30 s of exercise. The changes (Δ) in [PCr], [P_i], [ADP] and pH across the protocol were then calculated as the difference between end-exercise and baseline values. [PCr], [P_i] and [ADP] were expressed as absolute concentrations and as a percentage change relative to resting baseline (i.e., 100%). The overall rate of change for [PCr], [P_i], [ADP] and pH was calculated as the difference between end-exercise and baseline values divided by T_{lim}. EMG was average rectified and normalised to the first 30 s of each trial (aEMG). For analysis, T_{lim} values obtained from visits 5-8 were used. Visits 9-12 were used to overlay EMG and RPE responses to ^{31}P-MRS data.

Statistics

Differences in T_{lim}, baseline and end-exercise aEMG and muscle [PCr], [Pi], [ADP], and pH between control limbs (i.e., Leg_1 vs. Leg_2) were assessed using paired samples *t*-tests. A two-way repeated measures ANOVA (time x condition) was employed to test for differences in the profiles of muscle [PCr], [P_i], [ADP] and pH, aEMG (using 30 s mean values), and RPE (using 120 s mean values). Where the ANOVA revealed a significant main or interaction effect, post-hoc tests were completed using a Bonferroni correction. For calculation of effect size, partial eta squared (η^2) was used for omnibus tests. Cohen's *d* was used to calculate the effect size for paired *t*-tests and post-hoc comparisons. Where sphericity was violated, a greenhouse-geisser correction factor was applied. For all tests, results were considered statistically significant when $P<0.05$. Data are presented as means ± SD unless otherwise indicated. All statistical analyses were conducted using IBM SPSS Statistics version 24.

RESULTS

There was no difference in T_{lim} during the Leg_{1CON} (396 ± 105 s) and Leg_{2CON} (385 ± 104 s) protocols (P>0.05, d=0.10, figure 2). Moreover, there were no differences in [PCr], [P_i], [ADP], pH (table 1, figure 3), aEMG amplitude (table 2, figure 5) and RPE (figure 6) between Leg_{1CON} and Leg_{2CON} at any time (all P>0.05). Compared to Leg_{2CON}, T_{lim} was reduced by 19% when Leg_2 was preceded by exhaustive exercise in Leg_1 following the consumption of PL (Leg_{2CON}: 385 ± 104 s vs. $Leg_{2PL\text{-}CONTRA}$: 311 ± 92 s, P<0.01, d=0.76, figure 2).

Effect of ACT on single-leg exercise tolerance and contralateral leg fatigue

There was no difference in T_{lim} between the Leg_{1CON} (396 ± 105 s), Leg_{1ACT} (402 ± 101 s) and Leg_{1PL} (390 ± 106 s) conditions (P>0.05, η^2=0.07, figure 2). Both $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ T_{lim} were significantly lower compared to Leg_{2CON} (P<0.05, η^2=0.71, figure 2). However, there was no difference in T_{lim} between $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ (311 ± 92 s vs. 324 ± 85 s, respectively, d=0.15, P>0.05, figure 2).

Muscle metabolic measurements

The [PCr], [Pi], [ADP] and pH profiles are illustrated in figure 3 for Leg_{1PL} and Leg_{1ACT} and in figure 4 for Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$, respectively. There were no significant differences in [PCr], [Pi], [ADP] or pH measured at any time points between Leg_{1CON} and Leg_{2CON} (P>0.05, table 1, figure 3). Similarly, there were no differences in end-exercise [PCr] (Leg_{2CON}: 16.0 ± 3.0, $Leg_{2PL\text{-}CONTRA}$: 16.1 ± 2.4, $Leg_{2ACT\text{-}CONTRA}$: 15.7 ± 2.6 mM, η^2=0.13), [ADP] (Leg_{2CON}: 57.8 ± 20.7, $Leg_{2PL\text{-}CONTRA}$: 56.4 ± 16.8, $Leg_{2ACT\text{-}CONTRA}$: 55.3 ± 17.8 μM, η^2=0.09) and pH (Leg_{2CON}: 6.83 ± 0.15, $Leg_{2PL\text{-}CONTRA}$: 6.83 ± 0.20, $Lbeg_{2ACT\text{-}CONTRA}$: 6.80 ± 0.15, η^2=0.05) between the Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions (P>0.05, table 1, figure 4). However, end-exercise [Pi] was significantly lower in $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ compared to Leg_{2CON} (Leg_{2CON}: 21.8 ± 3.7, $Leg_{2PL\text{-}CONTRA}$:

18.8 ± 4.1, $Leg_{2ACT\text{-}CONTRA}$: 18.7 ± 3.9 mM, P=0.04, η^2=0.89, table 1, figure 4). Baseline [PCr] was significantly higher (36.6 ± 2.1 vs. 33.2 ± 3.2 vs. 33.2 ± 3.1 mM, P<0.0001, η^2=3.04), and [Pi] (Pi: 3.96 ± 0.7 vs. 5.2 ± 1.1 vs. 5.2 ± 1.0 mM, P<0.01, η^2=2.13) and [ADP] (ADP: 5.8 ± 1.2 vs. 11.4 ± 4.3 vs. 11.4 ± 4.5 μM, P<0.01, η^2=2.55, table 1, figure 4) were significantly lower, in Leg_{2CON} when compared to $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$, respectively. The rates of change for [Pi] (0.05 ± 0.01 vs. 0.05 ± 0.02 vs. 0.05 ± 0.02 mM/s, P>0.05, η^2=0.10), [PCr] (-0.06 ± 0.02 vs. -0.06 ± 0.04 vs. -0.06 ± 0.03 mmol/s, P>0.05, η^2=0.11), [ADP] (0.15 ± 0.09 vs. 0.17 ± 0.10 vs. 0.15 ± 0.09 μM/s, P>0.05, η^2=0.17) and pH (P>0.05, η^2=0.08) were not different between the Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions, respectively.

Electromyography (n=10)

aEMG amplitude of *m*. vastus lateralis rose significantly from the first minute of exercise to end-exercise in all conditions (figure 5; P<0.01, η^2=3.8). However, there were no differences in aEMG between Leg_{1CON}, Leg_{1PL} and Leg_{1ACT} at T_{lim} (Leg_{1CON}: 229 ± 54, Leg_{1PL}: 224 ± 43, Leg_{1ACT}: 238 ± 51%, P=0.69, η^2=0.09, table 2, figure 5). End-exercise aEMG in Leg_{2CON} was also similar to $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$, respectively (Leg_{2CON}: 234 ± 52, $Leg_{2PL\text{-}CONTRA}$: 226 ± 58, $Leg_{2ACT\text{-}CONTRA}$: 242 ± 52%, P=0.69, η^2=0.20, table 2, figure 5). However, absolute aEMG was elevated at the start of $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ exercise when compared to Leg_{2CON} (Leg_{2CON}: 0.04 ± 0.02, $Leg_{2PL\text{-}CONTRA}$: 0.05 ± 0.02, $Leg_{1ACT\text{-}CONTRA}$: 0.05 ± 0.02 mV, P<0.05, η^2=0.58, table 2, figure 5).

Ratings of perceived exertion

RPE increased in all trials following the onset of exercise (figure 6). However, there were no differences in RPE between Leg_{1CON}, Leg_{1PL} and Leg_{1ACT} at any time point (P>0.05, η^2=0.08, figure 6). The rate of rise and the end-exercise RPE were also similar during the Leg_{2CON} trial

compared with the $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ trials ($P>0.05$, $\eta^2=0.18$). However, at the onset of exercise, RPE was significantly higher in $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ when compared to Leg_{2CON} ($P<0.05$, $\eta^2=0.55$, figure 6). Specifically, during the first 2 min of exercise, there was a respective elevation in RPE of 14% and 13% in $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$, compared to Leg_{2CON} ($P<0.05$). There were no differences in RPE at any time points between $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ ($P>0.05$, $\eta^2=0.21$, figure 6).

DISCUSSION

The principal original finding of this study was that, while time to task failure was lower during severe-intensity single-leg knee extensor exercise after the completion of prior fatiguing exercise in the contralateral leg, this effect was not mitigated by acute ACT ingestion. We found no differences in the rates of change or end-exercise values for skeletal muscle activation (via EMG), metabolic perturbation (via ^{31}P-MRS) and perception of effort (via RPE) during exercise after prior contralateral leg fatigue following ACT and PL ingestion. Moreover, there were also no differences in time to task failure (i.e., T_{lim}), skeletal muscle activation, metabolic perturbation and RPE during single-leg exercise without the completion of prior fatiguing exercise in the contralateral leg following ACT and PL ingestion. These findings do not support our experimental hypotheses and suggest that 1 g of acute ACT ingestion does not improve time to task failure, skeletal muscle activation, metabolic perturbation or perceived exertion during single-leg severe-intensity knee extensor exercise completed with or without prior fatiguing exercise by the contralateral leg. Collectively, these results contribute to our understanding of fatigue development during exercise, performed with or without prior contralateral leg exercise, and in the presence or absence of ACT ingestion.

In the present study, T_{lim} in the $Leg_{2PL\text{-}CONTRA}$ protocol was shorter than the Leg_{2CON} protocol, indicative of an earlier task failure after completing exhaustive exercise in the contralateral leg compared to no prior fatiguing contralateral leg exercise. This observation is consistent with some (i.e., 3, 14, 22, 29, 33, 41), but not all (i.e., 16, 21, 34, 42, 45), previous studies reporting greater fatigue development after prior contralateral or non-local muscle fatigue. While the neuromuscular bases of contralateral fatigue development have yet to be fully resolved (23), there is evidence to suggest that greater central fatigue makes an important contribution to this phenomenon (3). In the current study, RPE was higher at baseline and over the initial stages of the $Leg_{2PL\text{-}CONTRA}$ test compared to the Leg_{2CON} test, leading to an earlier attainment of peak RPE and T_{lim}, consistent with previous observations (3) and the notion that afferent feedback may contribute to increased pain and effort sensation (1, 20). Amann and colleagues (3) reported a lower EMG response at task failure and reduced peripheral fatigue development after prior contralateral leg fatigue. Although the EMG amplitude was not different at task failure in the current study between the Leg_{2CON} and $Leg_{2PL\text{-}CONTRA}$ tests, baseline EMG was elevated in the $Leg_{2PL\text{-}CONTRA}$ condition, presumably due to isometric stabilisation, leading to the earlier attainment of the same peak EMG amplitude. The greater muscle activation in the non-exercising contralateral leg during the baseline 'resting' period in the $Leg_{2PL\text{-}CONTRA}$ condition was accompanied by lower muscle [PCr], and higher muscle [Pi] and [ADP], compared to the Leg_{2CON} condition. Since there were no differences in muscle [PCr] and [ADP] at T_{lim}, and since the rates of change in [PCr] and [ADP] were not different between the Leg_{2CON} and $Leg_{2PL\text{-}CONTRA}$ tests, the muscle [PCr] nadir and [ADP] peak were attained earlier in the $Leg_{2PL\text{-}CONTRA}$ test. These observations cohere with reports that the end-exercise values of muscle [PCr], [ADP] and pH are consistent when several bouts of exhaustive exercise of differing duration are completed within the severe-intensity domain (7, 44), and when T_{lim} is altered via prior passive heating of the legs (6) or by hyperoxic gas inhalation (44).

323 Interestingly, however, and despite a higher baseline muscle [Pi] in the $Leg_{2PL\text{-}CONTRA}$ condition
324 compared to the Leg_{2CON} condition, muscle [Pi] was lower at the point of task failure in the
325 $Leg_{2PL\text{-}CONTRA}$ test. These novel observations suggest that the ergolytic effect of prior
326 contralateral fatigue may be related, at least in part, to a limitation in the attainment of peak
327 intramuscular [Pi].
328
329 It is unclear why prior contralateral leg fatigue limited the attainment of peak [Pi] in the $Leg_{2PL\text{-}CONTRA}$
330 condition compared to the Leg_{2CON} condition, whereas the peak [ADP] and the nadir in
331 pH and [PCr] were not different between these conditions. However, our observations of a
332 limited peak perturbation of muscle [Pi], but not pH, [PCr] and [ADP], when group III/IV
333 muscle afferent feedback would be expected to be elevated via prior contralateral fatigue (3),
334 are in accord with studies from another group who observed greater peak perturbation of
335 muscle [Pi], but not pH, [PCr] and [ADP], when group III/IV muscle afferent feedback was
336 abolished via lumbar intrathecal administration of fentanyl (8, 11, 12). Taken together, these
337 complementary observations suggest that intramuscular phosphorous-containing metabolites
338 and substrates may not respond in a uniform manner to manipulations in skeletal muscle group
339 III/IV afferent feedback and that muscle [Pi] might be a more sensitive marker of peripheral
340 fatigue development. However, it should be acknowledged that, since inter-test variability is
341 greater for contracting skeletal muscle [Pi] than for pH, [PCr] and [ADP] (15), further research
342 is required to verify these observations.
343
344 Although the completion of prior single-leg fatiguing exercise lowered T_{lim} during subsequent
345 exercise in the contralateral leg in the current study, there were no differences between the
346 $Leg_{2ACT\text{-}CONTRA}$ and $Leg_{2PL\text{-}CONTRA}$ conditions in T_{lim}, RPE, or muscle activation and
347 phosphorous-containing metabolites and substrates. Similarly, and also in contrast to our

hypothesis, acute ACT ingestion did not alter T_{lim}, RPE, or muscle activation, pH, [PCr], [ADP] or [Pi], during single-leg severe-intensity knee extensor exercise completed without prior fatiguing exercise in the contralateral leg, with these responses being similar between the Leg_{1CON}, Leg_{1PL} and Leg_{1ACT} conditions. These findings conflict with reports that acute ACT consumption can improve exercise performance by increasing work output for the same level of pain and effort sensation (19, 30) and by increasing muscle activation (31, 32).

Experimental Considerations

The lack of an ergogenic effect of ACT administration in the current study might be due to differences in the ACT administration procedure compared to previous studies reporting improved performance and delayed neuromuscular fatigue development (19, 30, 31, 32). In the present study, ACT was ingested 45 min prior to the start of the Leg_{1ACT} test, which immediately transitioned into the $Leg_{2ACT\text{-}CONTRA}$ protocol that was the primary focus of the current study. Since peak plasma [ACT] is attained ~60 min post oral ACT ingestion (4, 17), we elected to administer ACT such that peak plasma [ACT] was expected to coincide with the onset of the $Leg_{2ACT\text{-}CONTRA}$ protocol rather than the Leg_{1ACT} protocol. This might account for the lack of an ergogenic effect of ACT during the Leg_{1ACT} protocol compared to other studies that administered ACT 60 min prior to the performance trial (19, 30, 31, 32). Therefore, we cannot exclude the possibility that earlier ACT ingestion (18), at the same or a greater dose (19, 30), might have resulted in improved single-leg severe-intensity exercise tolerance. However, it should also be noted that inter-study differences in participant characteristics (i.e., training status, motivation and responsiveness to analgesic medication) may have contributed to the differences in ergogenicity observed following ACT ingestion between the current study and some previous studies (19, 30, 31, 32).

In addition to differences in the ACT dosing procedure, the lack of an ergogenic effect of ACT administration in the current study might be linked to the nature of the fatiguing exercise test administered. Our subjects completed continuous single-leg severe-intensity knee extensor exercise until task failure with no pre-determined end-point (i.e., an 'open loop' exercise test). This differs from situations in which ACT ingestion has been reported to be ergogenic, such as completion of a fixed distance (16.1 km) time trial (30), a fixed number of maximal effort repetitions (19, 31), or a fixed duration of maximal effort (32), all of which have a predetermined end point (i.e., a 'closed loop' exercise task). Moreover, since exercise-induced pain sensation is positively associated with exercise intensity (5, 13), and since ACT ingestion is suggested to be ergogenic by mitigating pain sensation (19, 30), this might account for the lack of improvement in performance in the longer duration, continuous severe-intensity exercise test we employed compared to the improved exercise performance that has been reported during maximal-intensity exercise (19, 31, 32). With regard to contralateral fatigue development, we cannot exclude the possibility that ACT might have been effective at attenuating the effects of prior single-leg fatigue on T_{lim} during subsequent exercise if a greater degree of contralateral fatigue had been attained. For example, T_{lim} was lowered by 19% in $Leg_{2PL\text{-}CONTRA}$ compared to Leg_{2CON} in the current study, whereas Amann et al. (3) reported a much larger (49%) reduction in T_{lim} following contralateral limb fatigue, which would have provided greater scope for an ergogenic effect with ACT ingestion. Moreover, since RPE is higher and T_{lim} is shorter at the same relative exercise intensity when a larger muscle mass is recruited (35), it is possible that ACT ingestion might have improved T_{lim} during exercise after prior fatigue had a larger muscle mass been recruited in either the initial or the subsequent fatiguing exercise task. Further research is required to assess the exercise settings in which ACT administration is more or less likely to be ergogenic.

In conclusion, the completion of prior single-leg fatiguing exercise compromised exercise tolerance during subsequent exercise in the contralateral leg. This ergolytic effect of prior contralateral leg fatigue was accompanied by elevated baseline RPE, muscle activation and [ADP], and lower baseline [PCr], leading to the earlier attainment of peak (RPE, muscle activation and [ADP]) or nadir (muscle [PCr]) values in these variables, and the attainment of a submaximal end-exercise [Pi]. However, acute ACT ingestion was not effective at lowering perceived exertion, increasing muscle activation or intramuscular perturbation or enhancing T_{lim} during single-leg severe-intensity exercise completed with or without prior fatigue in the contralateral leg. These findings do not support an ergogenic effect of analgesia, at least using the ACT administration and exercise testing procedures employed in the current study.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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593 **Tables with their headings**

Table1. Muscle metabolic responses in Leg_{1CON}, Leg_{1PL}, Leg_{1ACT}, Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions.

	Leg_{1CON}	**Leg_{1PL}**	**Leg_{1ACT}**	**Leg_{2CON}**	**$Leg_{2PL\text{-}CONTRA}$**	**$Leg_{2ACT\text{-}CONTRA}$**
Muscle metabolic response						
[PCr]						
Baseline PCr (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	92 ± 5*	93 ± 4*
PCr at 120 s (%)	70 ± 8	70 ± 8	71 ± 47	71 ± 8	62 ± 9*	63 ± 7*
End-exercise PCr (%)	42 ± 9	41 ± 9	41 ± 8	44 ± 8	45 ± 7	44 ± 8
Rate of change PCr (mmol/s)	-0.06 ± 0.01	-0.06 ± 0.03	-0.06 ± 0.02	-0.05 ± 0.03	-0.06 ± 0.04	-0.06 ± 0.03
[Pi]						
Baseline Pi (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	125 ± 24*	126 ± 23*
Pi at 120 s (%)	310 ± 66	313 ± 71	306 ± 62	312 ± 66	316 ± 70	318 ± 64
End-exercise Pi (%)	590 ± 149	590 ± 137	594 ± 156	588 ± 177	459 ± 110*	460 ± 109*
Rate of change Pi (mmol/s)	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
[ADP]						
Baseline ADP (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	200 ± 78*	201 ± 77*
ADP at 120 s (%)	404 ± 161	415 ± 183	400 ± 148	412 ± 170	538 ± 176	516 ± 154*
End-exercise ADP (%)	1028 ± 386	1036 ± 421	1046 ± 409	1024 ± 401	980 ± 316	978 ± 312
Rate of change ADP (µmol/s)	0.15 ± 0.08	0.15 ± 0.09	0.14 ± 0.07	0.15 ± 0.09	0.17 ± 0.10	0.15 ± 0.09
pH						
Baseline pH	7.04 ± 0.01	7.03 ± 0.02	7.05 ± 0.04	7.04 ± 0.03	7.04 ± 0.03	7.05 ± 0.02
pH at 120 s	6.96 ± 0.09	6.94 ± 0.07	6.92 ± 0.08	6.95 ± 0.08	6.93 ± 0.10	6.94 ± 0.08
End-exercise pH	6.77 ± 0.18	6.76 ± 0.15	6.76 ± 0.16	6.83 ± 0.15	6.83 ± 0.20	6.80 ± 0.15

PL, placebo; ACT, acetaminophen; EMG, electromyography; PCr, Phosphocreatine; Pi, Inorganic Phosphate; ADP, Adenosine diphosphate; *significantly different from $Leg_{2CON,}$ $P<0.05$

594

595 **Tables**

Table2. Electromyography (EMG) responses of *m.* vastus lateralis in Leg_{1CON}, Leg_{1PL}, Leg_{1ACT}, Leg_{2CON}, $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ conditions.

	Leg_{1CON}	Leg_{1PL}	Leg_{1ACT}	Leg_{2CON}	$Leg_{2PL\text{-}CONTRALATERAL}$	$Leg_{2ACT\text{-}CONTRALATERAL}$
Neuromuscular function						
Baseline EMG_{RMS} amplitude (mV)	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02*	0.05 ± 0.02*
End-exercise EMG_{RMS} amplitude (%)	229 ± 54	224 ± 43	238 ± 51	234 ± 52	226 ± 58	242 ± 52
EMG_{RMS} amplitude at 120 s (%)	150 ± 27	160 ± 25	166 ± 26	158 ± 29	155 ± 32	158 ± 34

PL, placebo; ACT, acetaminophen; EMG, electromyography; RMS, root mean square; *significantly different from Leg_{2CON}, $P<0.05$. Data are from 10 subjects.

596

Legends to figures

Figure 1

Protocol schematic. Visits 1-4 were completed within a replica of the MRI scanner. Subjects completed a single-leg incremental test on the left leg (visit 1, Leg_1) and right leg (visit 2, Leg_2). Subjects then completed a familiarisation session on visits 3 and 4 which comprised a single-leg severe-intensity constant work rate (CWR) test to task failure with Leg_1, Leg_2 and a crossover test where the Leg_1 protocol was repeated and immediately followed by the Leg_2 protocol (interspersed by 10 min of passive recovery). During visits 5 and 6, subjects completed the Leg_1 and Leg_2 protocols, respectively, without oral consumption of any capsules. On visits 7 and 8, subjects commenced the crossover test, 45 mins following the consumption of 1 g maltodextrin (PL) and 45 mins following the consumption of 1 g ACT. Visits 5-8 were completed within the bore of an MRI scanner for assessment of intramuscular phosphorous substrates and metabolites and then replicated within a replica of the MRI scanner (visits 9-12) to assess muscle electromyography (EMG) and ratings of perceived exertion (RPE). The dashed vertical lines represent the limit of tolerance (i.e., T_{lim}) for each trial and/or leg, respectively.

Figure 2

Exercise tolerance (time to task failure, s) in Leg_{1CON}, Leg_{2CON}, Leg_{1PL}, $Leg_{2PL\text{-}CONTRA}$, Leg_{1ACT} and $Leg_{2ACT\text{-}CONTRA}$ conditions. Data are presented as mean ± SD *significantly different from Leg_{1CON}, Leg_{2CON}; Leg_{1PL} and Leg_{1ACT} (P<0.05).

Figure 3

Intramuscular phosphocreatine concentration ([PCr]; panel A), inorganic phosphate concentration ([Pi]; panel B), adenosine diphosphate ([ADP]; panel C) and pH (panel D) during

622 severe-intensity, single-leg knee-extensor exercise in the left leg following PL ingestion
623 (Leg_{1PL}, filled circles) and ACT (Leg_{1ACT}, clear circles) ingestion. Data are expressed as group
624 mean ± SE.
625
626 *Figure 4*
627 Intramuscular phosphocreatine concentration ([PCr]; panel A), inorganic phosphate
628 concentration ([Pi]; panel B), adenosine diphosphate ([ADP]; panel C) and pH (panel D) during
629 severe-intensity, single-leg knee-extensor exercise in the right control leg (Leg_{2CON}, open
630 triangles) and in the right leg following prior exhaustive exercise in the left leg after PL
631 ingestion ($Leg_{2PL\text{-}CONTRA}$, filled circles) and ACT ($Leg_{2ACT\text{-}CONTRA}$, clear circles) ingestion. Data
632 are expressed as group mean ± SE. *T_{lim} significantly different from $Leg_{2PL\text{-}CONTRA}$ and
633 $Leg_{2ACT\text{-}CONTRA}$ (P<0.05); #significantly different from $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$
634 (P<0.05).
635
636 *Figure 5*
637 Surface electromyography (EMG) of the *m.*vastus lateralis muscle during severe-intensity,
638 single-leg knee-extensor exercise in Leg_{1PL} (filled circles) and Leg_{1ACT} (clear circles) (panel
639 A), and in the right control leg (Leg_{2CON}, open triangles), and in Leg_2 following prior exhaustive
640 exercise in Leg_1 after PL ($Leg_{2PL\text{-}CONTRA}$, filled circles) and ACT ($Leg_{2ACT\text{-}CONTRA}$, clear circles)
641 ingestion (panel B). Mean values for average rectified EMG (aEMG) during each muscle
642 contraction were calculated and averaged over each 30-s period. Data are expressed as group
643 mean ± SE relative to the first 30 s of each trial. *T_{lim} significantly different from $Leg_{2PL\text{-}CONTRA}$
644 and $Leg_{2ACT\text{-}CONTRA}$ (P<0.05).
645
646 *Figure 6*

Ratings of perceived exertion (RPE) during severe-intensity, single-leg knee-extensor exercise of the left leg in Leg_{1PL} (filled circles) and Leg_{1ACT} (clear circles) (panel A), in the right control leg (Leg_{2CON}, open triangles), and in the right leg following prior exhaustive exercise in the left leg after PL ingestion ($Leg_{2PL\text{-}CONTRA}$, filled circles) and ACT ($Leg_{2ACT\text{-}CONTRA}$, clear circles) ingestion (panel B). Data are expressed as group mean ± SE. *T_{lim} significantly different from $Leg_{2PL\text{-}CONTRA}$ and $Leg_{2ACT\text{-}CONTRA}$ ($P<0.05$); $^{\#}$RPE significantly different from Leg_{2CON} ($P<0.05$).